IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: CARTER, Daniel C.

Serial No.: 09/616,962

Filed: July 14, 2000

For: MODIFIED SERUM ALBUMIN WITH REDUCED AFFINITY FOR NICKEL AND COPPER

Examiner: Sisson, Bradley L.

Art Unit: 1634

Docket #: P06652US0/BAS

#11/ Declaration
V. Bui
3/19/03

DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner for Patents
Washington, D.C.

S I R:

I, Dr. Daniel C. Carter, Ph.D., declare as follows:

1. I am the inventor of the above-identified application, and I have also been the named inventor in numerous other patents in the general field of protein crystallography included several relating specifically to albumin. I am thus well familiar with the field of the invention, and I am familiar with the conception and reduction to practice of the presently claimed invention.

2. The present invention relates to the discovery that truncated forms of human serum albumin will provide reduced affinity to undesirable trace metals such as nickel and copper. The invention is important in that the truncated albumins of the invention can perform the function of natural albumin yet at the same time reduce or eliminate the possibility of the formation of a nickel complex which is known to elicit a potentially life-threatening allergic reaction in some individuals. In addition, the present invention will producer a safer albumin product, such as a pharmaceutical additive, by further eliminating hypoallergenic concerns, and the product will also not have an undesirable color often caused by the presence of trace metals.

3. My invention, which is reflected in the current claims of the above application, was to provide a truncated version of human serum albumin wherein a single residue at the n-terminal end was removed from the final product. In addition, the invention included additional truncations, a mutation of the sequence at the His-3 position and elongation or insertion by one or more residues. All of these forms of the modified albumin of the invention are reflected in the current set of claims.

4. The claimed invention was conceived and reduced to practice prior to April 13, 2000 as reflected in the documents attached hereto. A copy of my invention disclosure record (attached hereto as Exhibit A with the dating removed) reflects the completion of the present invention, and this record is dated prior to April 13, 2000. As reflected in the disclosure, this invention record represents the complete invention as described in the present claims, and also that the invention had been conceived, tested and reduced to practice by the date of the invention. This invention record was reflected in the invention as described in the present application filed July 14, 2000.

5. Moreover, attached hereto is additional documentation showing the reduction to practice of the invention prior to April 13, 2000, namely that human serum albumin truncates in accordance with the invention were prepared and sequenced under my direction. The sequencing of the truncates of the invention is reflected in a copy of the raw data obtained during the protein sequencing of the truncates (attached hereto as Exhibit B with dates redacted), and these truncates in accordance with the claimed invention were prepared prior to April 13, 2000.

6. Further to the above raw data, a copy of a letter from my associate, Dr. Florian Ruker (attached hereto as Exhibit C, with dates redacted), also summarizes the results regarding the sequencing of the truncates of the claimed invention. Although the dates included in this letter have been redacted, these dates again confirm actual preparation of the claimed truncates and reduction to practice of the present invention prior to April 13, 2000.

7. In short, the presently claimed invention was conceived and reduced to practice prior to the critical date of April 13, 2000.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

3-7-03
Date

Dr. Daniel C. Carter, Ph.D.

EXHIBIT A

A.

The general field of the invention involves improvements in the metal binding properties of recombinant albumins, specifically recombinant human serum albumin. The applications of the improved rHSA include safer blood volume expanders, excipient and culture media applications.

B.

The development of the invention was made entirely by the NCP corporation independent of any grant or contract.

C.

The potential for rHSA as a safer volume expander, etc. is widely accepted. For example the production of albumin in yeast without the utilization of animal derived components yields plasma protein free from undesirable pathogens which are potentially present in all animal derived plasma protein products.

Prior art utilizes the human serum albumin sequence which represents the prototypical or major allotype of the human serum albumin sequence. Specifically this includes the widely known and accepted n-terminal amino acid sequence: n-DAHK-c (ref). The binding of copper and nickel to the n-terminal peptide of albumins has been known and studied for many years ( ). This site has been designated as functional binding location Site VI by Carter and Ho (1994). The sequence X-X-Histidine is key to the copper an nickel metal binding at this site and an additional requirement is the structural flexibility of the n-terminal polypeptide (ie., it cannot be structurally hindered).

At physiological pH, nickel and copper are bound with high affinity to this site (Ka)(ref). While this feature of the albumin molecule serves to protect the body from the potential damaging influences of the metal, the nickel complex with albumin is known to illicit a potentially life threatening allergic reaction to some individuals.

In the normal course of recombinant production of albumin and other proteins, trace amounts of metals, including nickel are a normal component of the culture media used in production. Consequently, a significant level of copper chelated by the n-terminal peptide of albumin during production as noted by the dark green and yellow coloration of crude rHSA.

D.

The preferred invention involves the truncation of the amino acid sequence of HSA by a single residue. This is sufficient to eliminate the nickel binding at this site. However, other embodiments of this invention will include: (1) additional truncation(s); (2) mutation of the sequence at His-3; and (4) elongation or insertion at the n-terminus by one or more residues (first demonstration).

Example:

Addition of the following amino acids to the n-terminus, XX, in the recombinant (NCP lot number) resulted in greatly reduced coloration of the purified recombinant albumin. Tis can be qualitatively demonstrated by the reduction in A666 for the invention vs the normal rHSA when produced and purified under otherwise identical conditions.

A.....

E. Alternative forms of the invention given above in section D.

F.
The advantages of the new invention are invlved in the elimination of the major high affinity copper and nickel site of human serum albumin. In the areas where the recombinant product either through expression in yeast, animals or plants may be exposed to significant amounts of copper or nickel, this invention can eliminate concerns over allergic reactions to nickel. In other applications where albumin may be utilized such as in cosmetics, shampoos, or additives to many other pharmaceuticals and non-pharmaceutical products, the resulting modifications further eliminate hypoallergenic concerns and can also improve the appearance (color) of the final product.

EXHIBIT B

N-terminale Proteinsequenzierung

von

D1, D2 und D3

PS 98069, 98070, 98071

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Analysenergebnis

Sequencer: ABI-Procise 492	*LAUF-Nr.* 98069
PROBE Rüker-D1 B	*Datum*
ID Prof. Rüker Inst. f. angewandte Mikrobiol./ Wien	*Tel.* 01-36006-6240

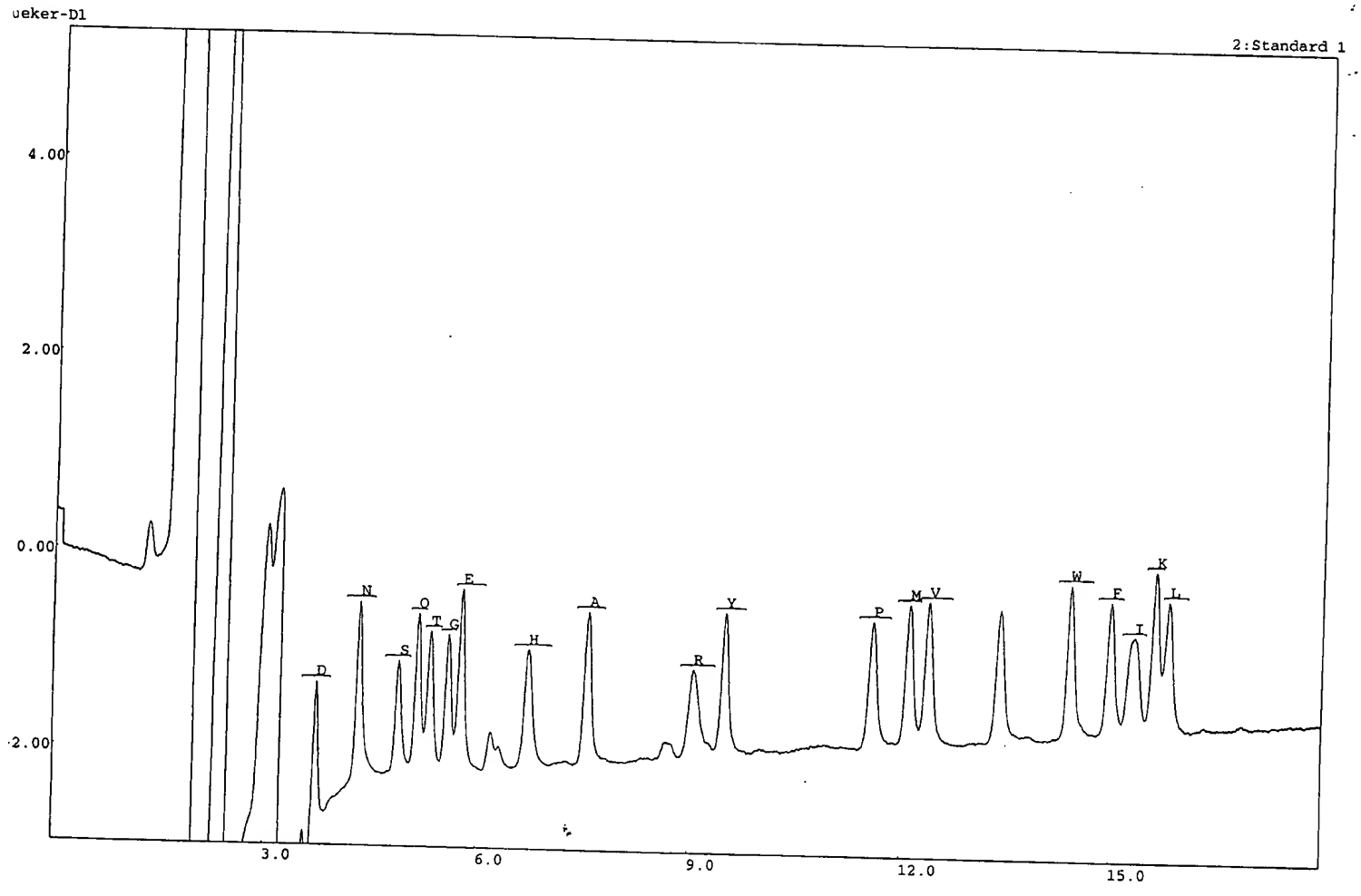
Peptid	Protein	PVDF	GF	BIOPR	BLOT	Menge	MW	I.Y. 17.4pM R.Y. %
☐	■	■	☐	☐	☐	≈50pM	≈23kD	

AS	Haupt-Sequenz	Neben-Sequenz	AS	Haupt-Sequenz	Neben-Sequenz	AS	Haupt-Sequenz	Neben-Sequenz
1	*E*		16			31		
2	*A*	*F*	17			32		
3	*E*	*D*	18			33		
4	*F*	*A*	19			34		
5	*D*	*E,H*	20			35		
6			21			36		
7			22			37		
8			23			38		
9			24			39		
10			25			40		
11			26			41		
12			27			42		
13			28			43		
14			29			44		
15			30			45		

Kommentar

2 Sequenzen, wobei die in etwas geringerer Menge vorkommende Sequenz um 2 AS kürzer ist als die Hauptsequenz. Nebensequenz ca. 1/5 d. Menge d. Hauptsequenz.

OP
B. Sa...



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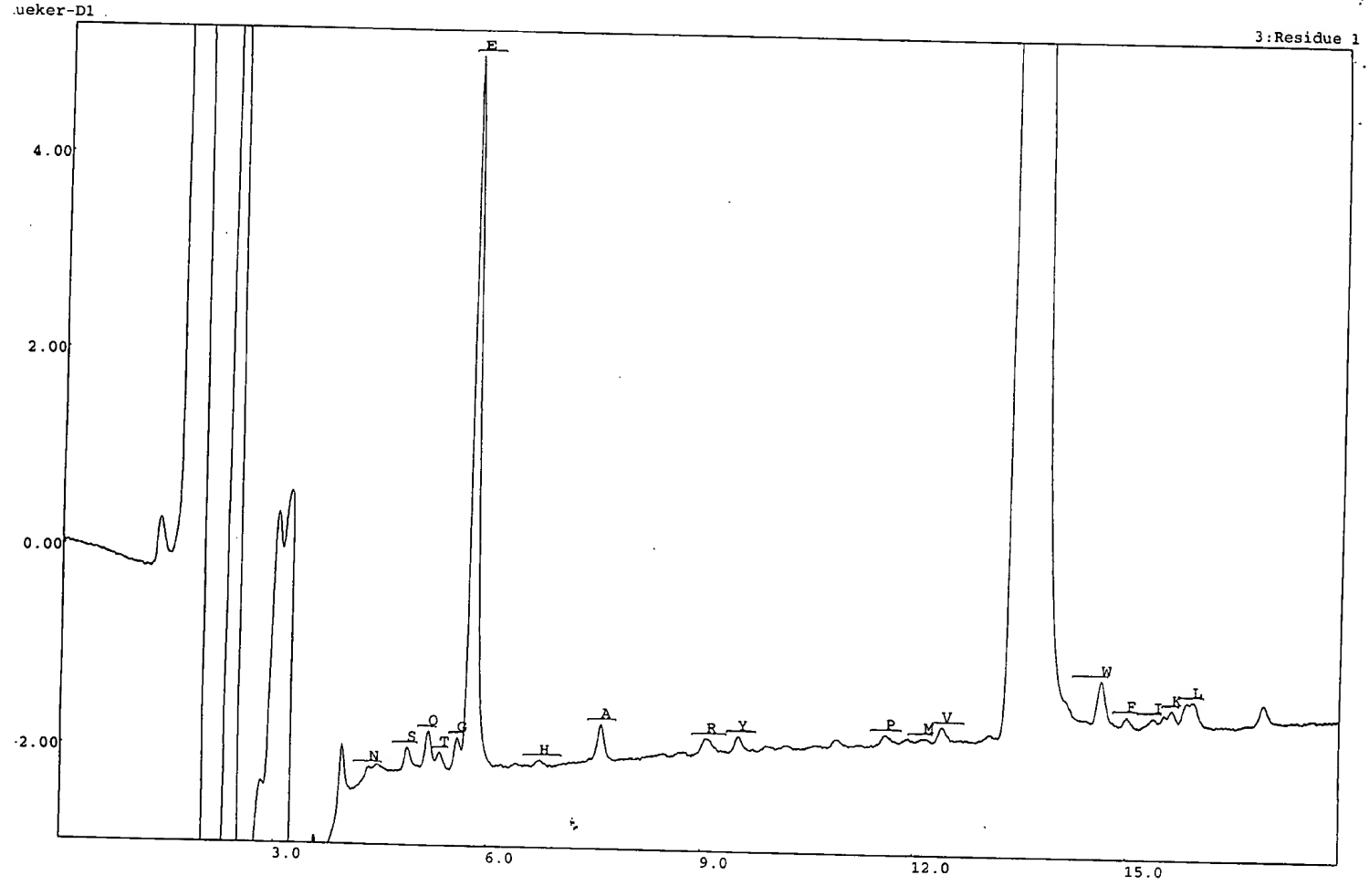

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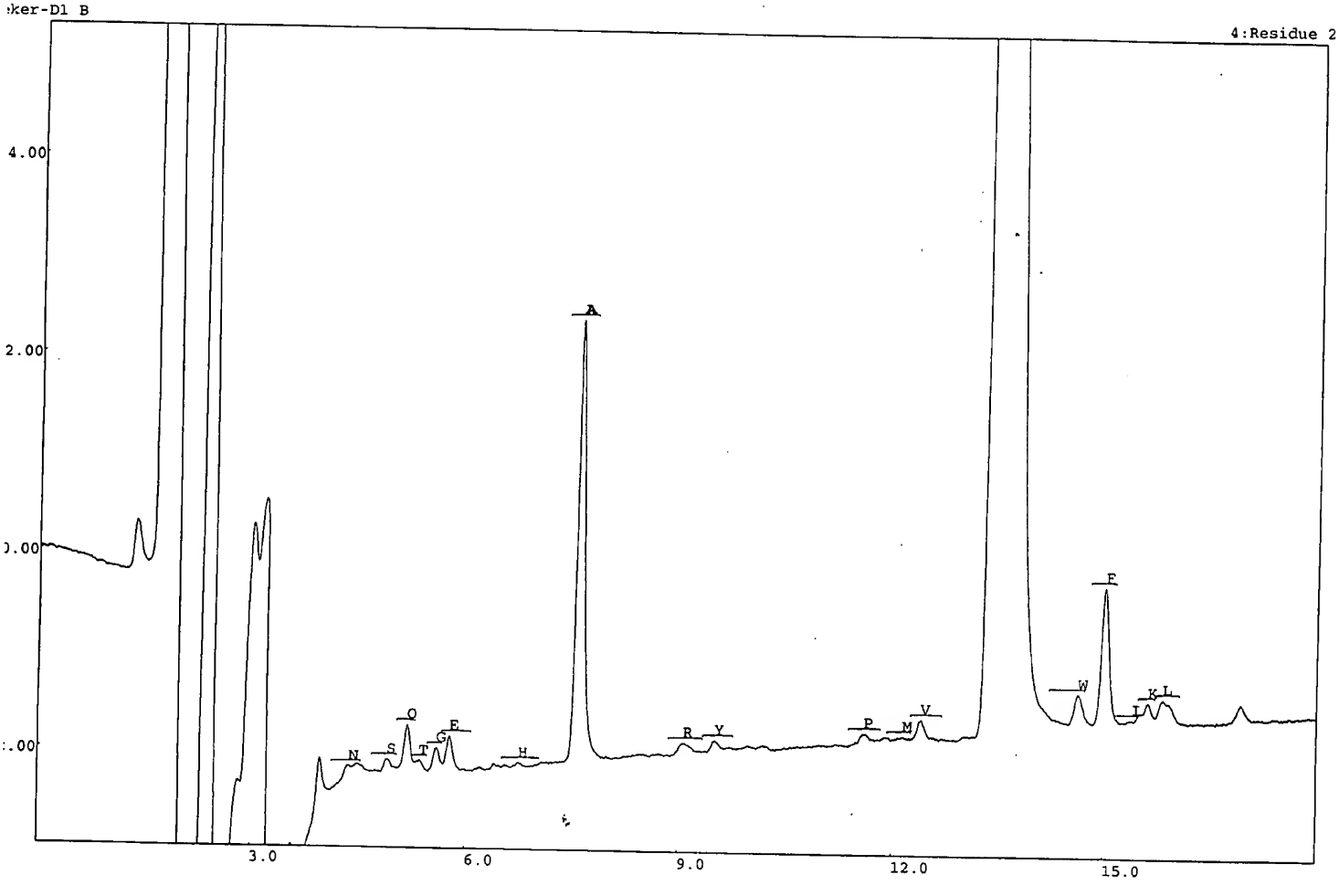

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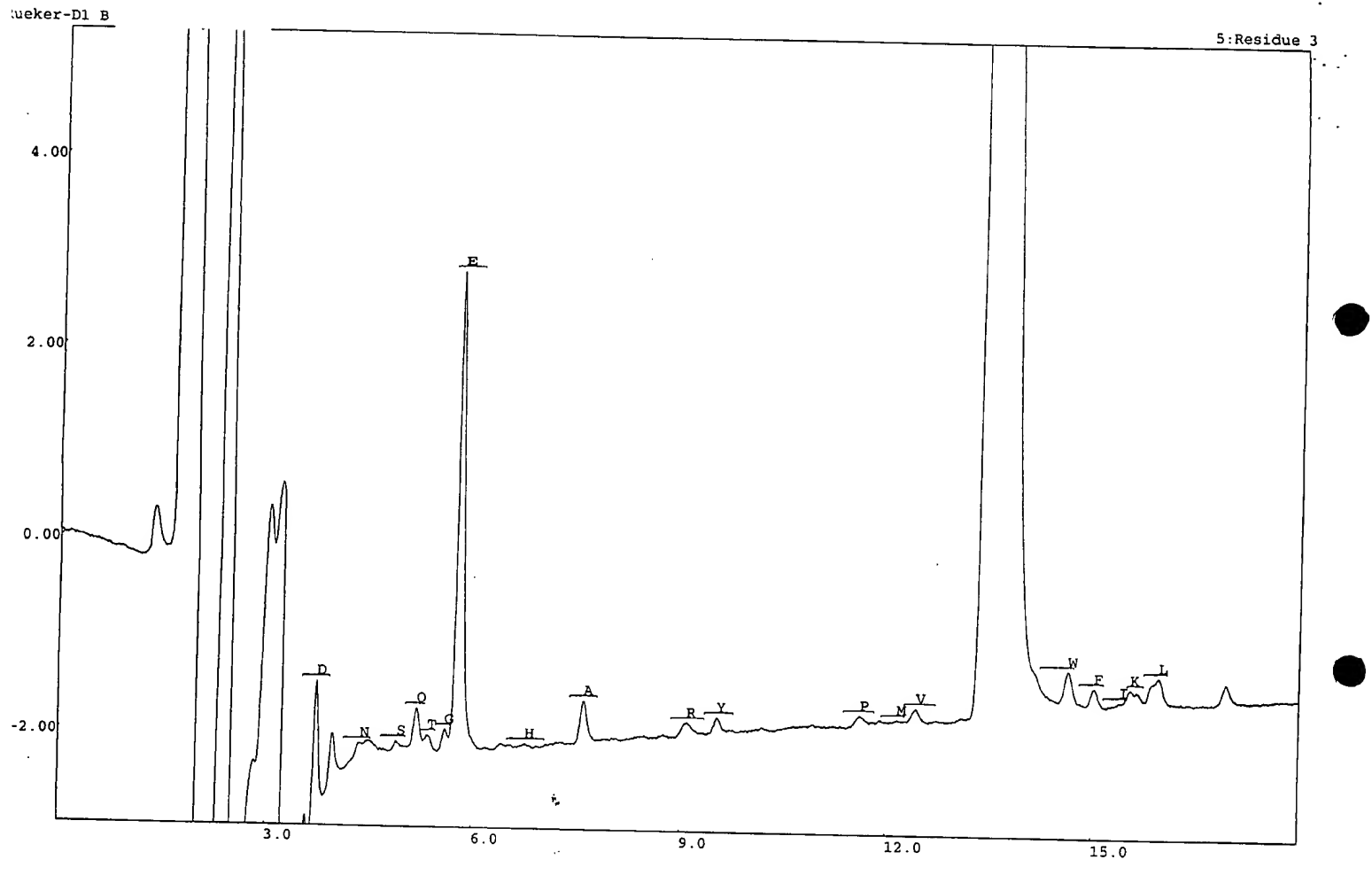

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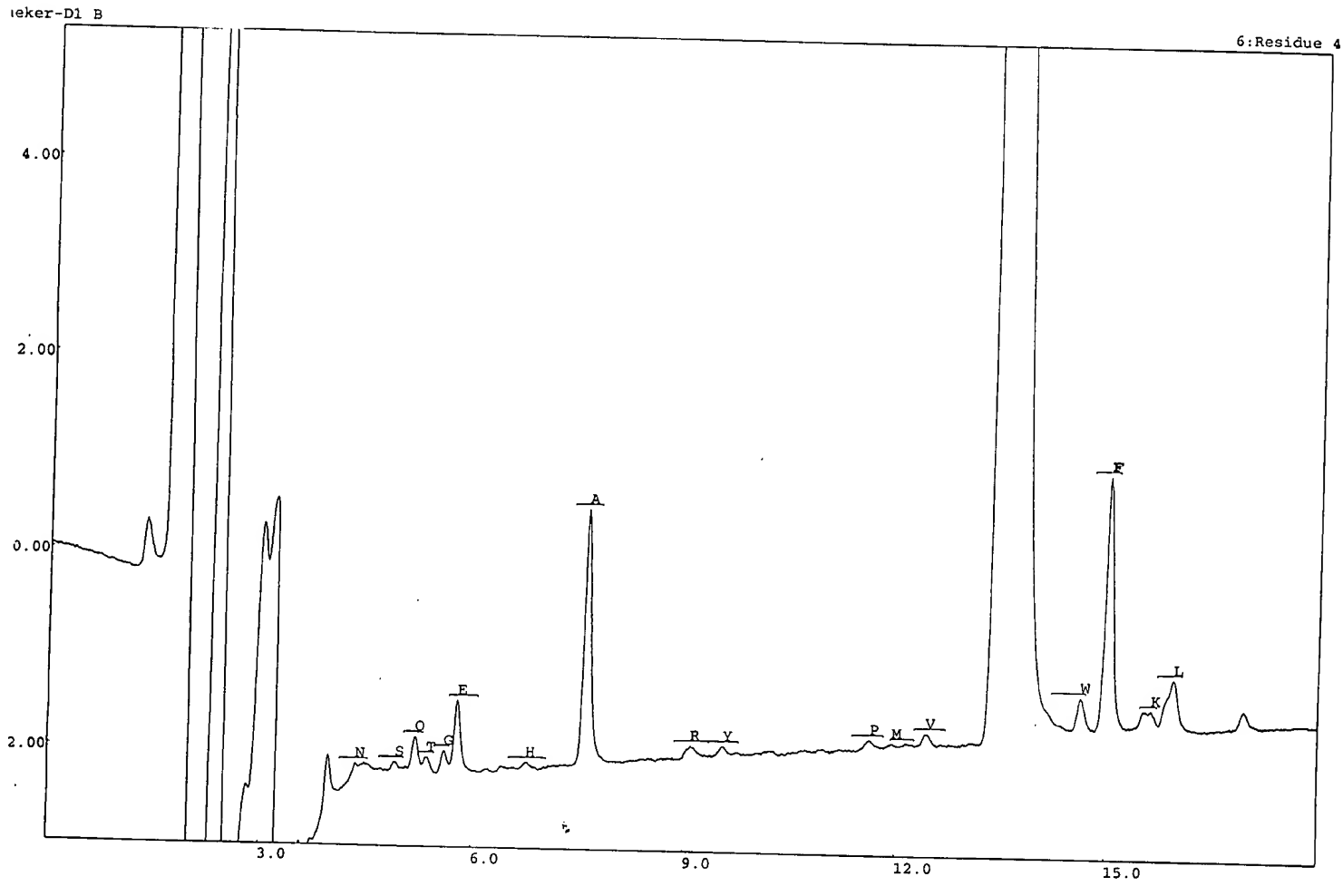

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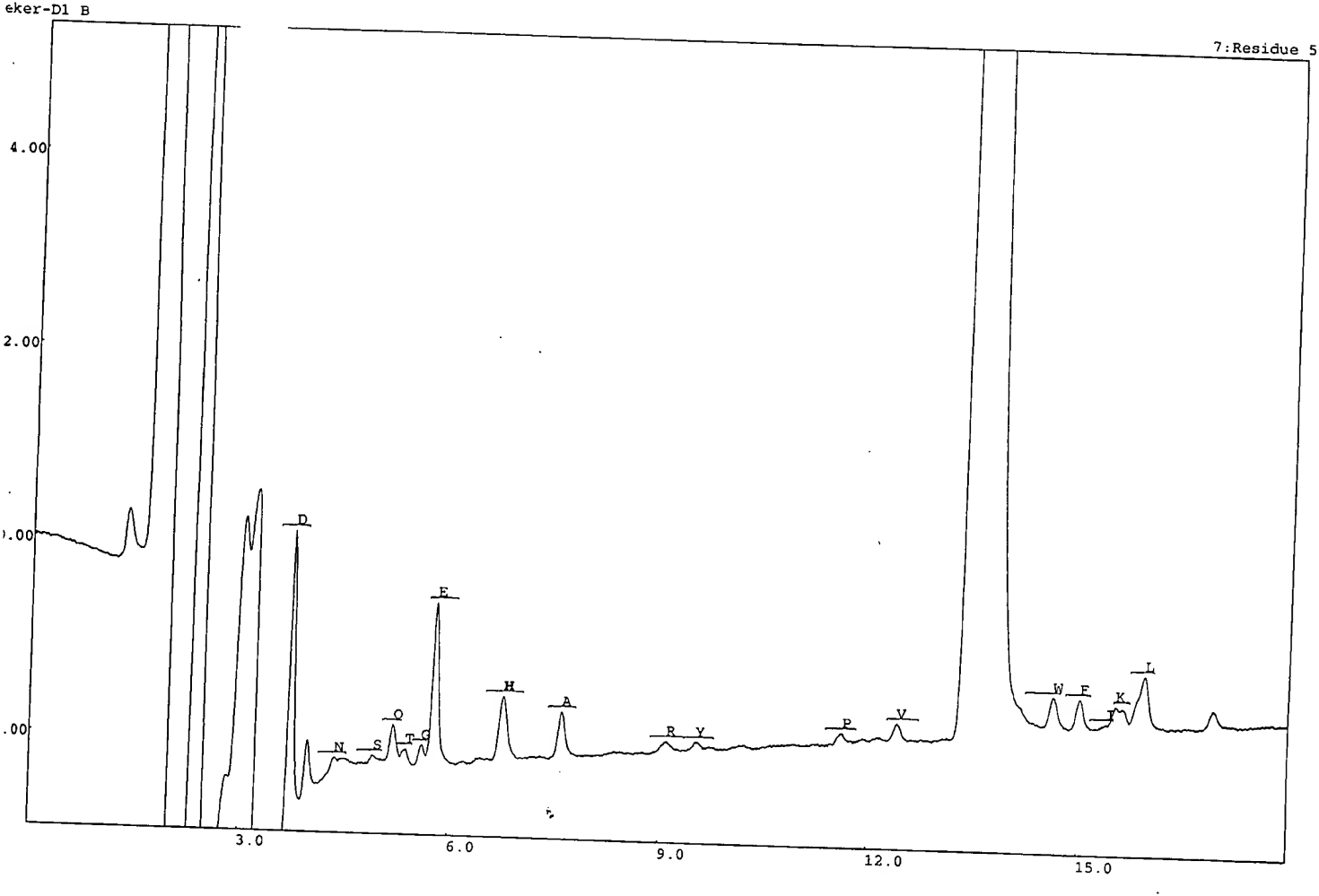

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Analysenergebnis

Sequencer: ABI-Procise 492	*LAUF-Nr.* 98070
PROBE : Rüker-D2 B	*Datum*
ID Prof. Rüker Inst. f. angewandte Mikrobiol./ Wien	*Tel.* 01-36006-6240

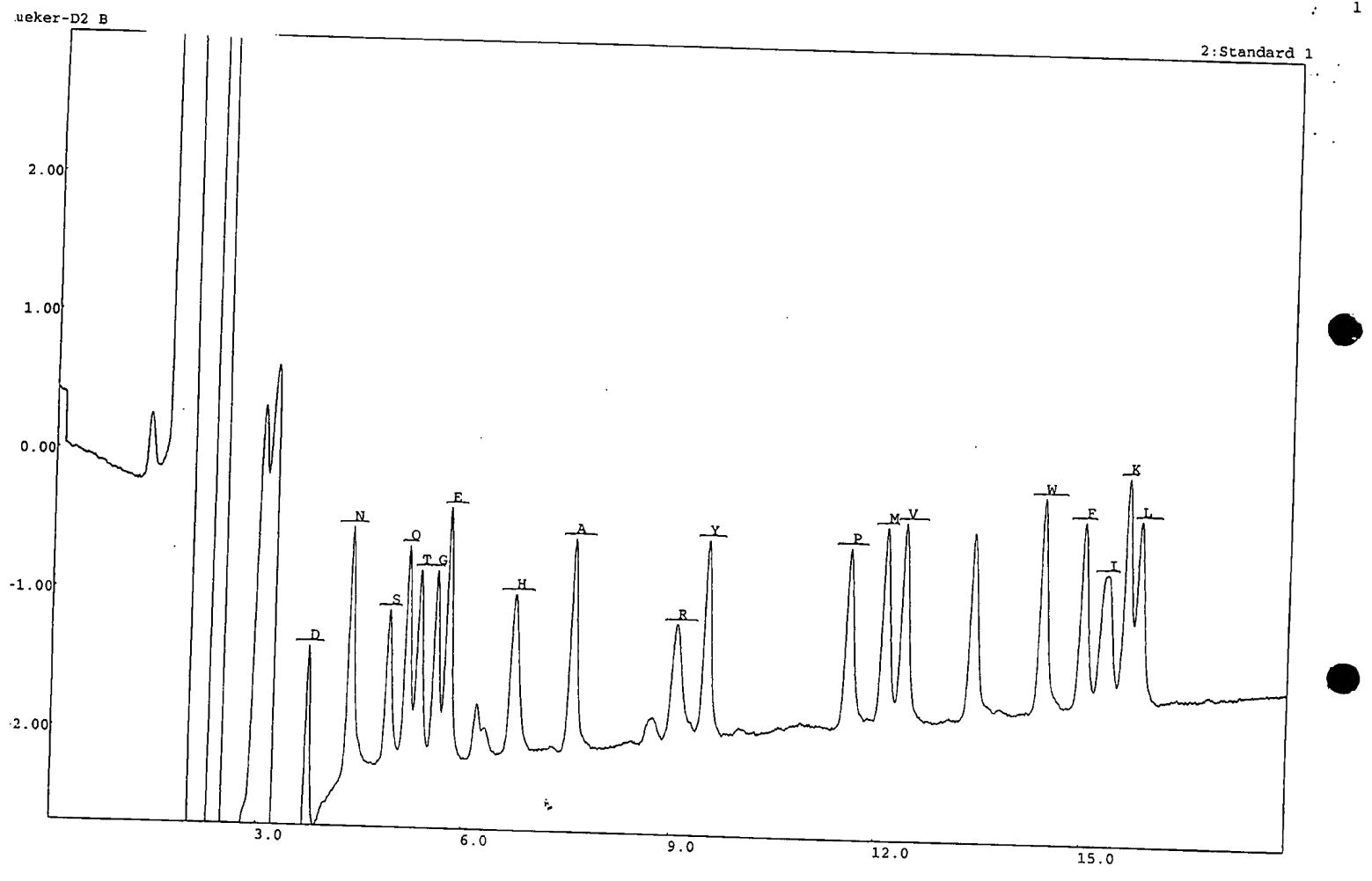
Peptid	Protein	PVDF	GF	BIOPR	BLOT	Menge	MW	I.Y 11pM R.Y %
☐	■	■	☐	☐	☐	≈50pM	≈22.5k D	

AS	Haupt- Sequenz	Neben	AS	Haupt- Sequenz	Neben	AS	Haupt- Sequenz	Neben-
1	***E***	*(L)*	16			31		
2	***F***	*A*	17			32		
3	***G***	*E*	18			33		
4	***K***	*F*	19			34		
5	***A***	*G*	20			35		
6			21			36		
7			22			37		
8			23			38		
9			24			39		
10			25			40		
11			26			41		
12			27			42		
13			28			43		
14			29			44		
15			30			45		

Kommentar

kleine Nebensequenz sichtbar, die um 2 AS kürzer ist als die Hauptsequenz.
Nebensequenz ca. 1/5 d. Menge d. Hauptsequenz.

OP



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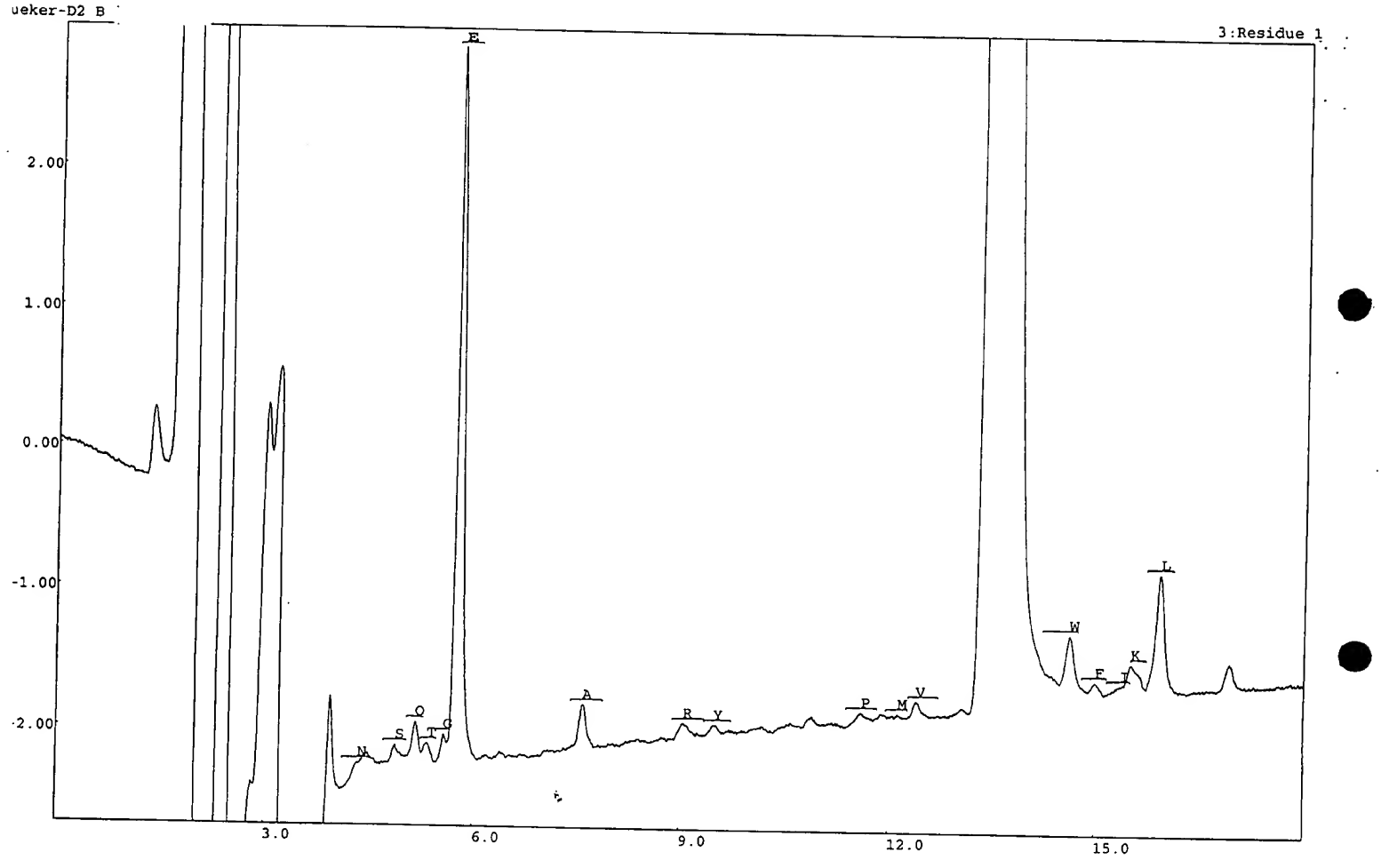

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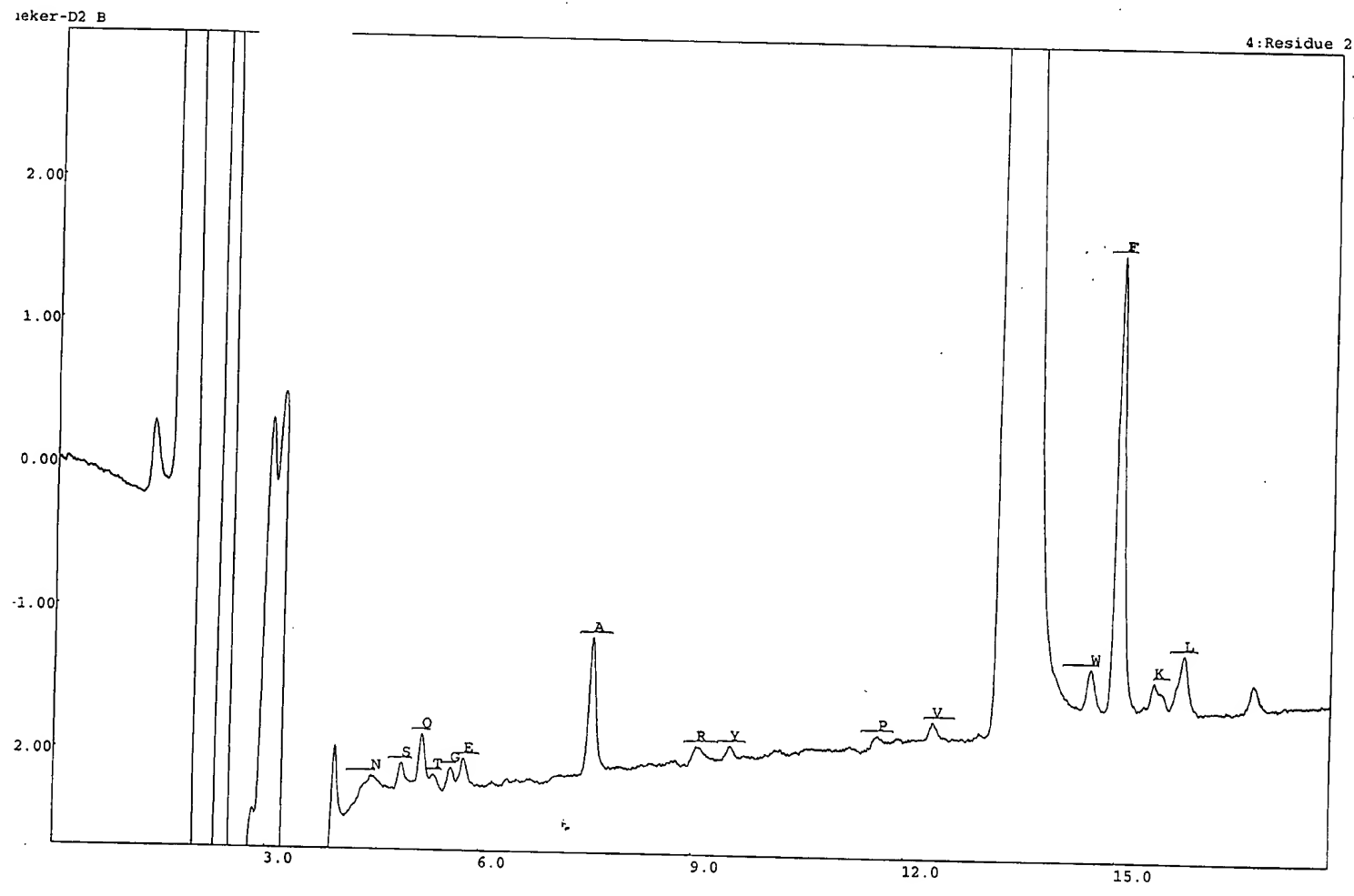

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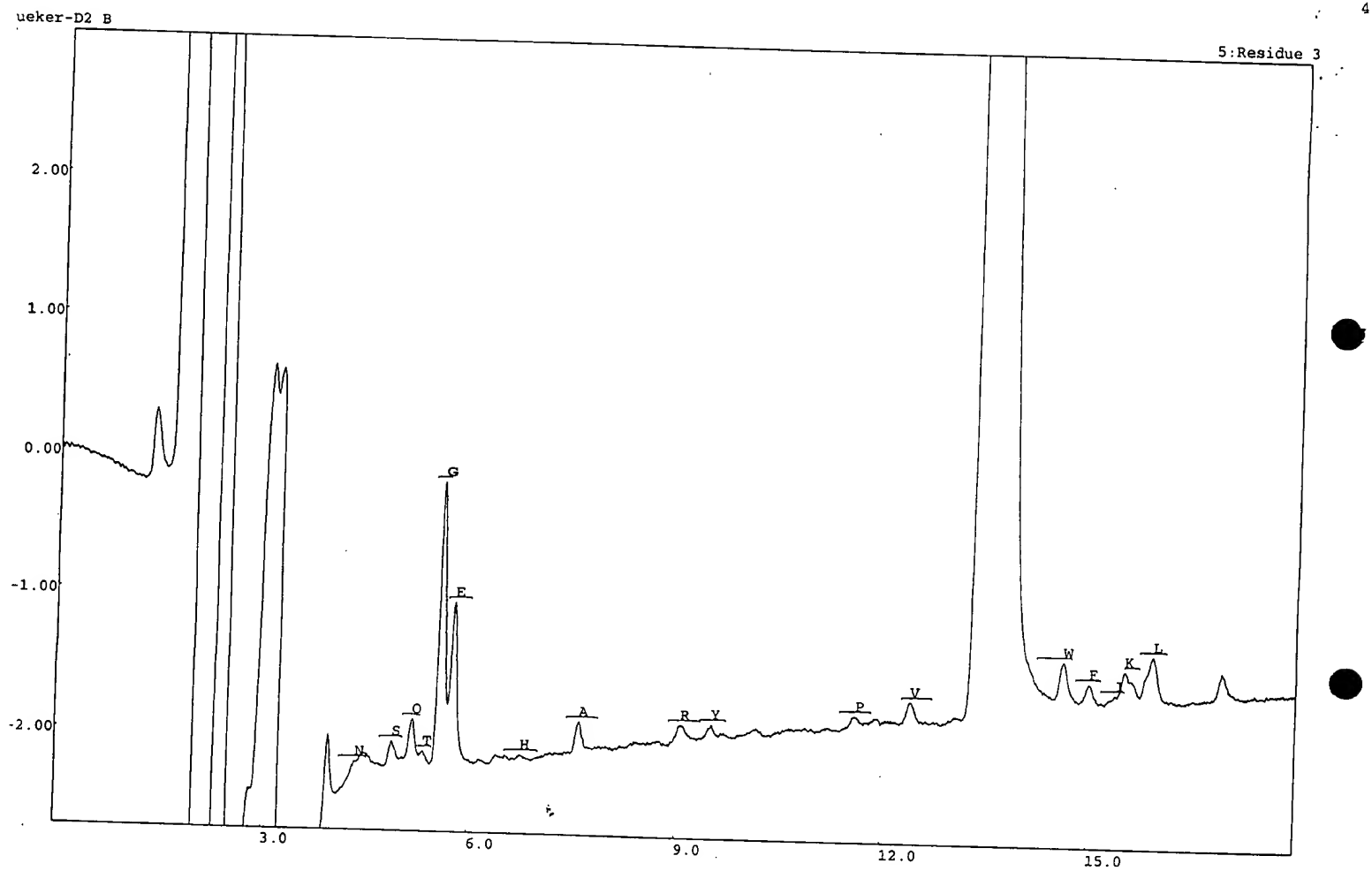

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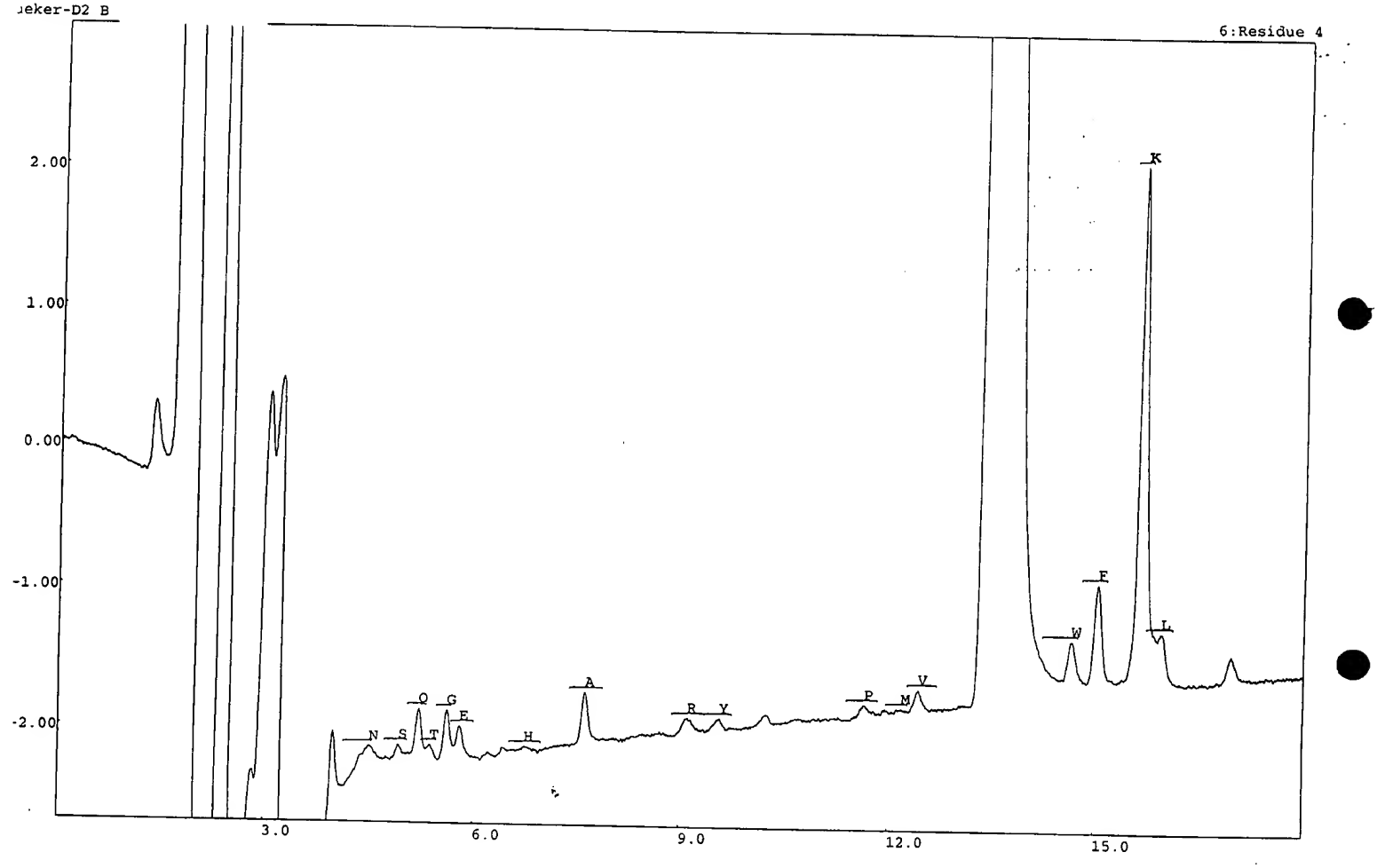
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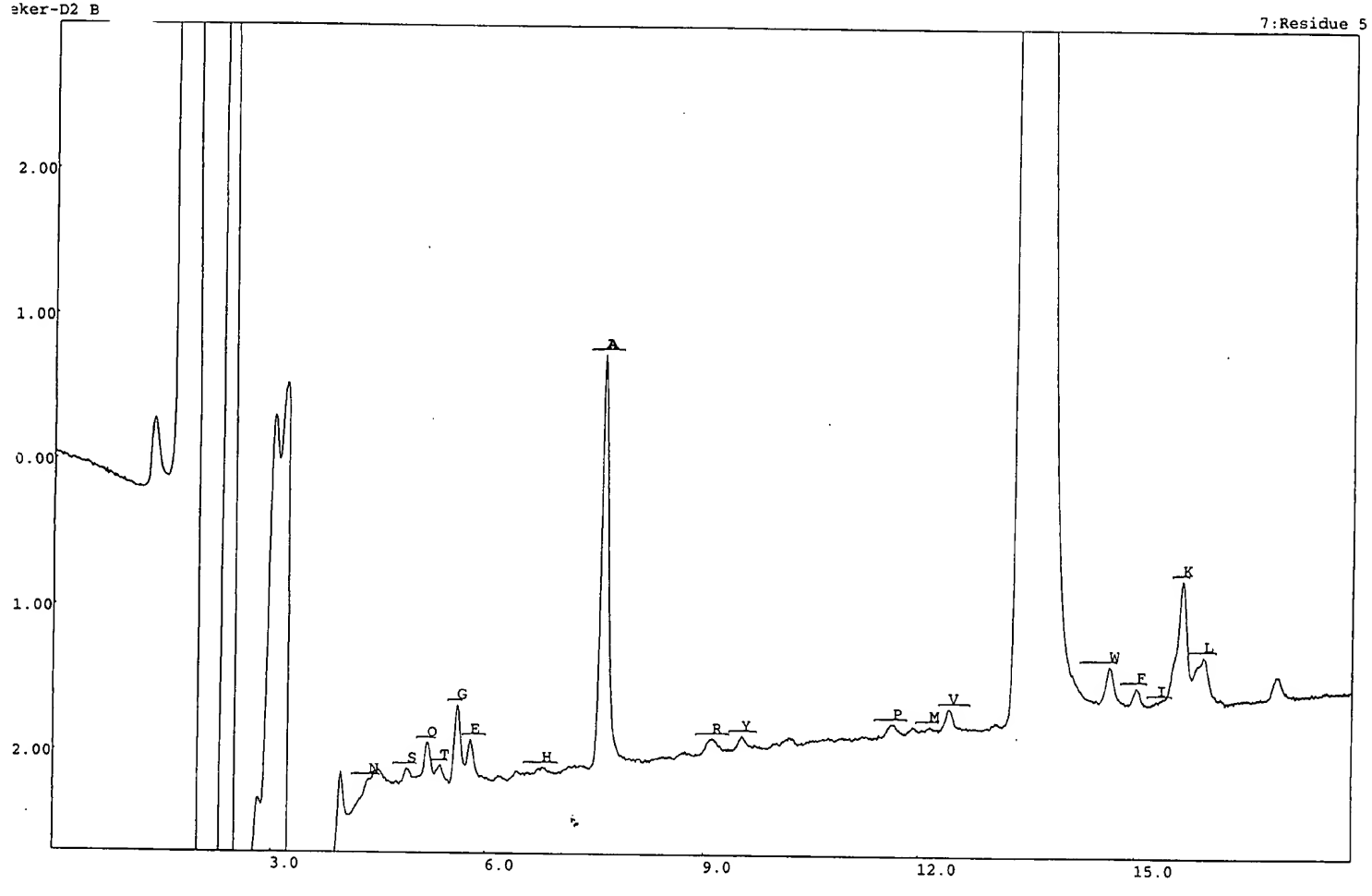
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PROBE: Rüker-D3 A	*Datum*
ID Prof. Rüker Inst. f. angewandte Mikrobiol./ Wien	*Tel.* 01-36006-6240

Peptid	Protein	PVDF	GF	BIOPR	BLOT	Menge	MW	I.Y. 11.3pM R.Y %
❑	■	■	❑	❑	❑	≈50pM	≈23.3k D	

AS	Haupt- Sequenz	Neben Sequenz	AS	Haupt- Sequenz	Neben Sequenz	AS	Haupt- Sequenz	Neben- Sequenz
1	*E*	*(L)*	16			31		
2	*A*	*F*	17			32		
3	*E*	*V,D*	18			33		
4	*F*	*E*	19			34		
5	*V*	*E*	20			35		
6			21			36		
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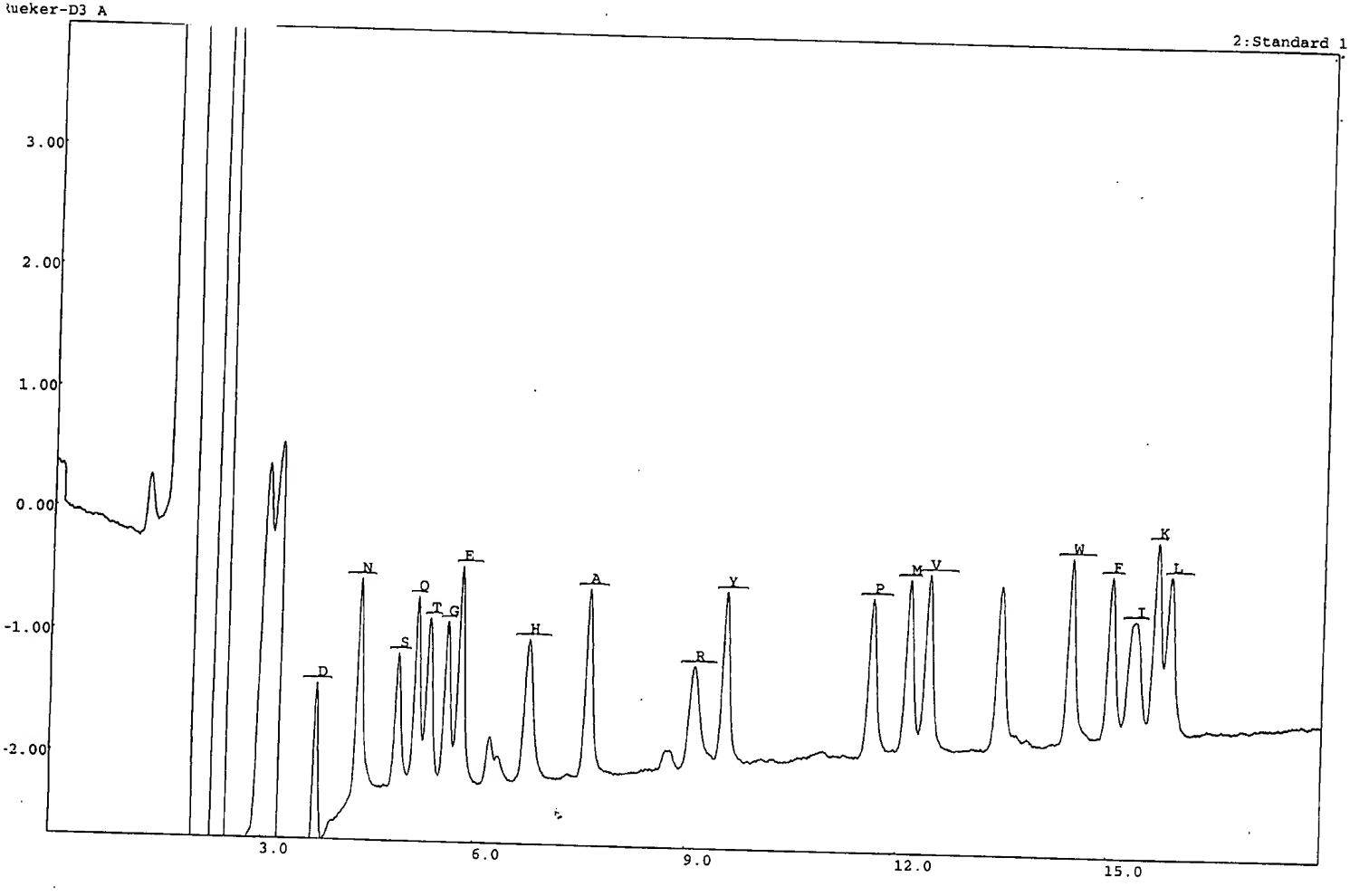
11			26			41		
12			27			42		
13			28			43		
14			29			44		
15			30			45		

Kommentar

kleine Nebensequenz sichtbar, die um 2 AS kürzer ist als die Hauptsequenz.
Nebensequenz ca. 1/3 d. Menge d. Hauptsequenz.

OP B. Sa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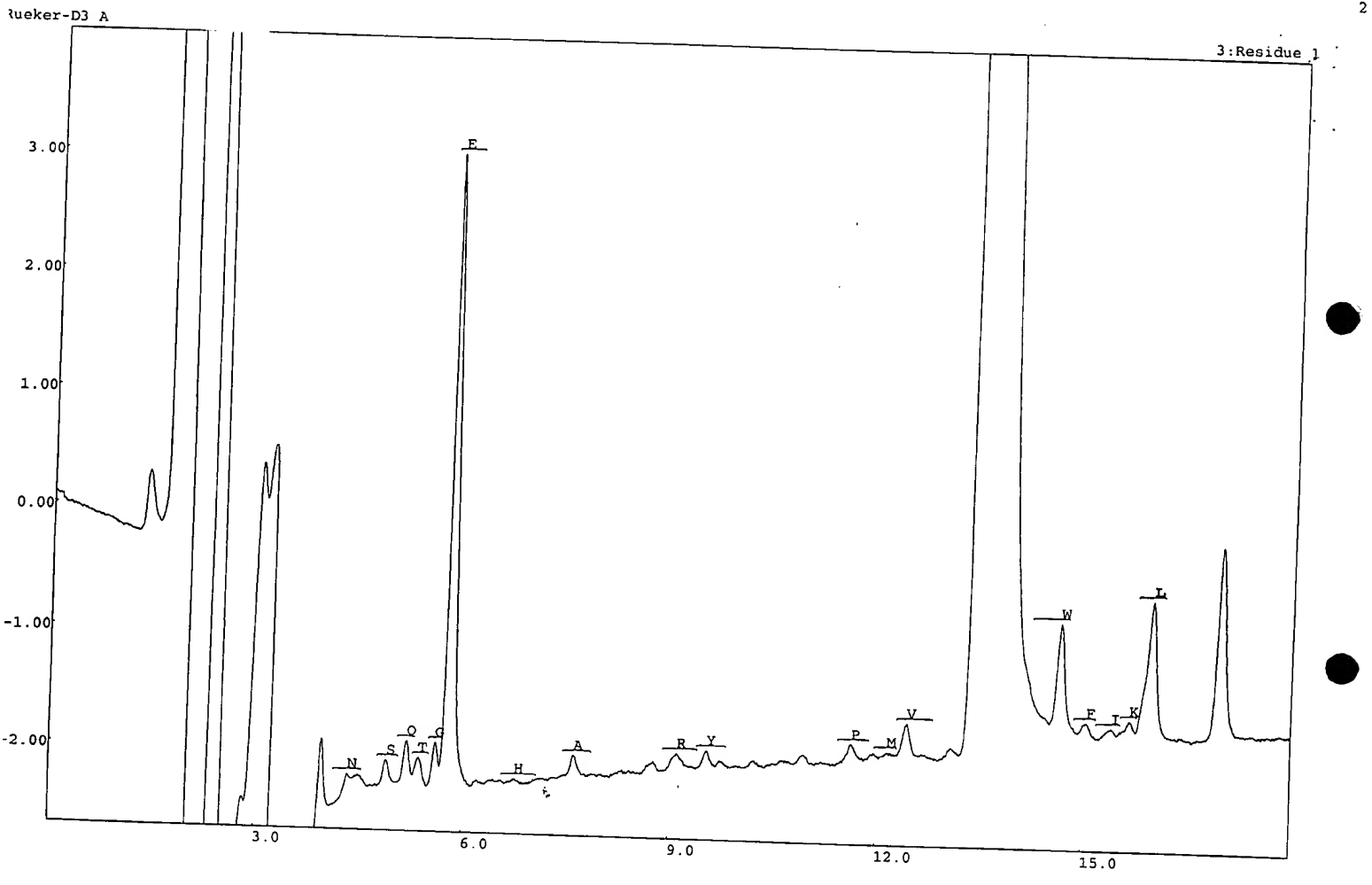
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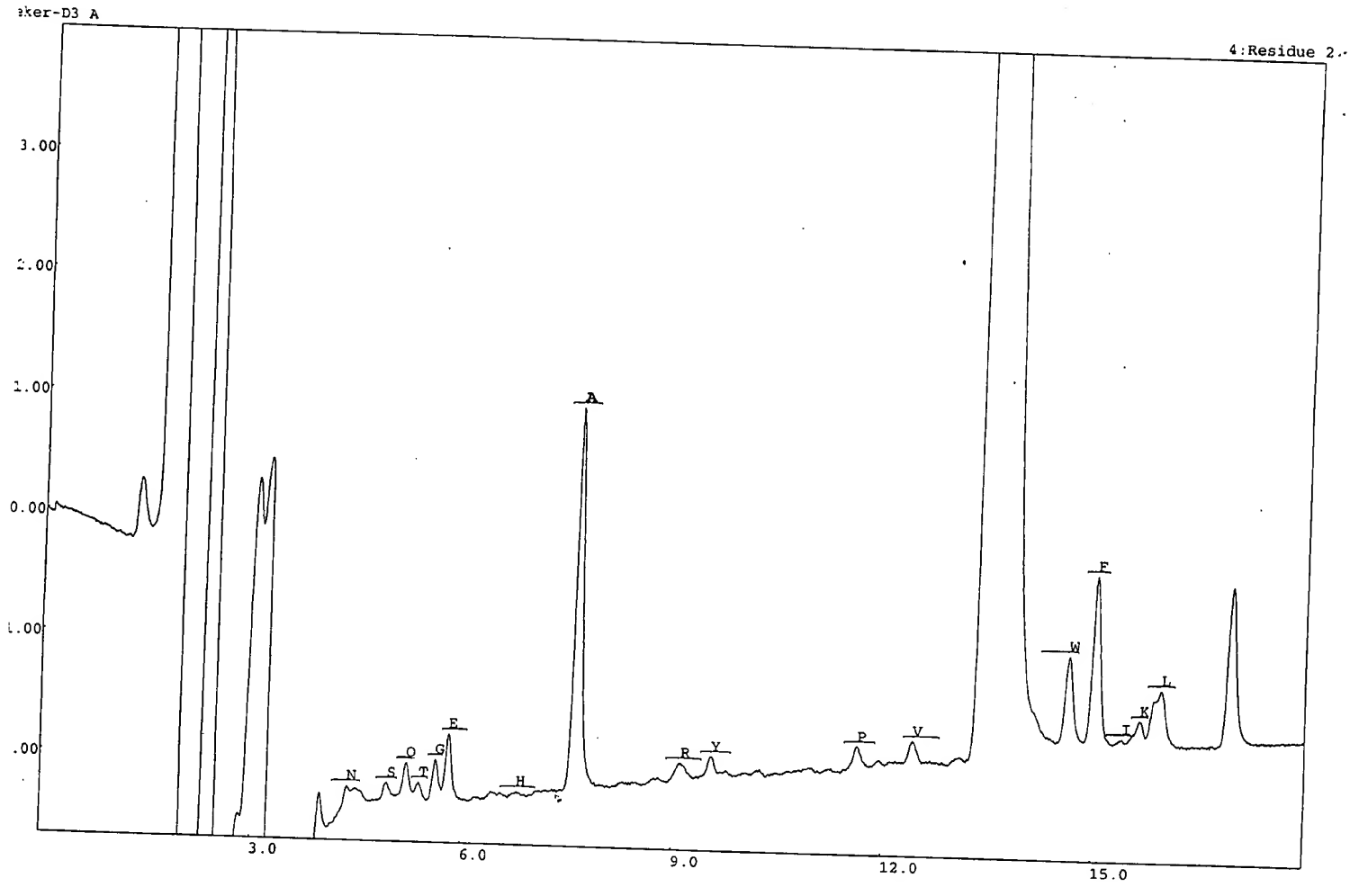

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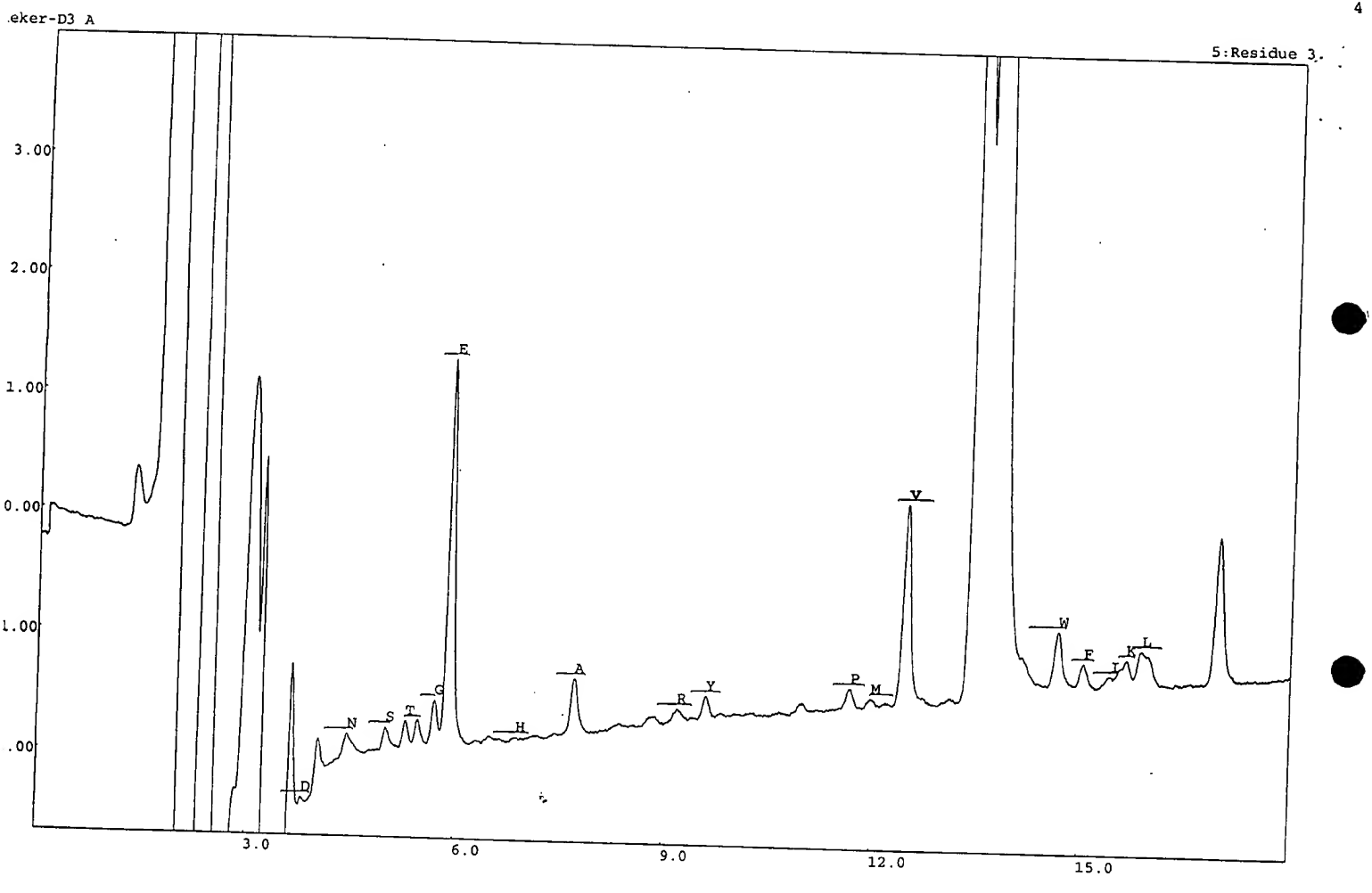

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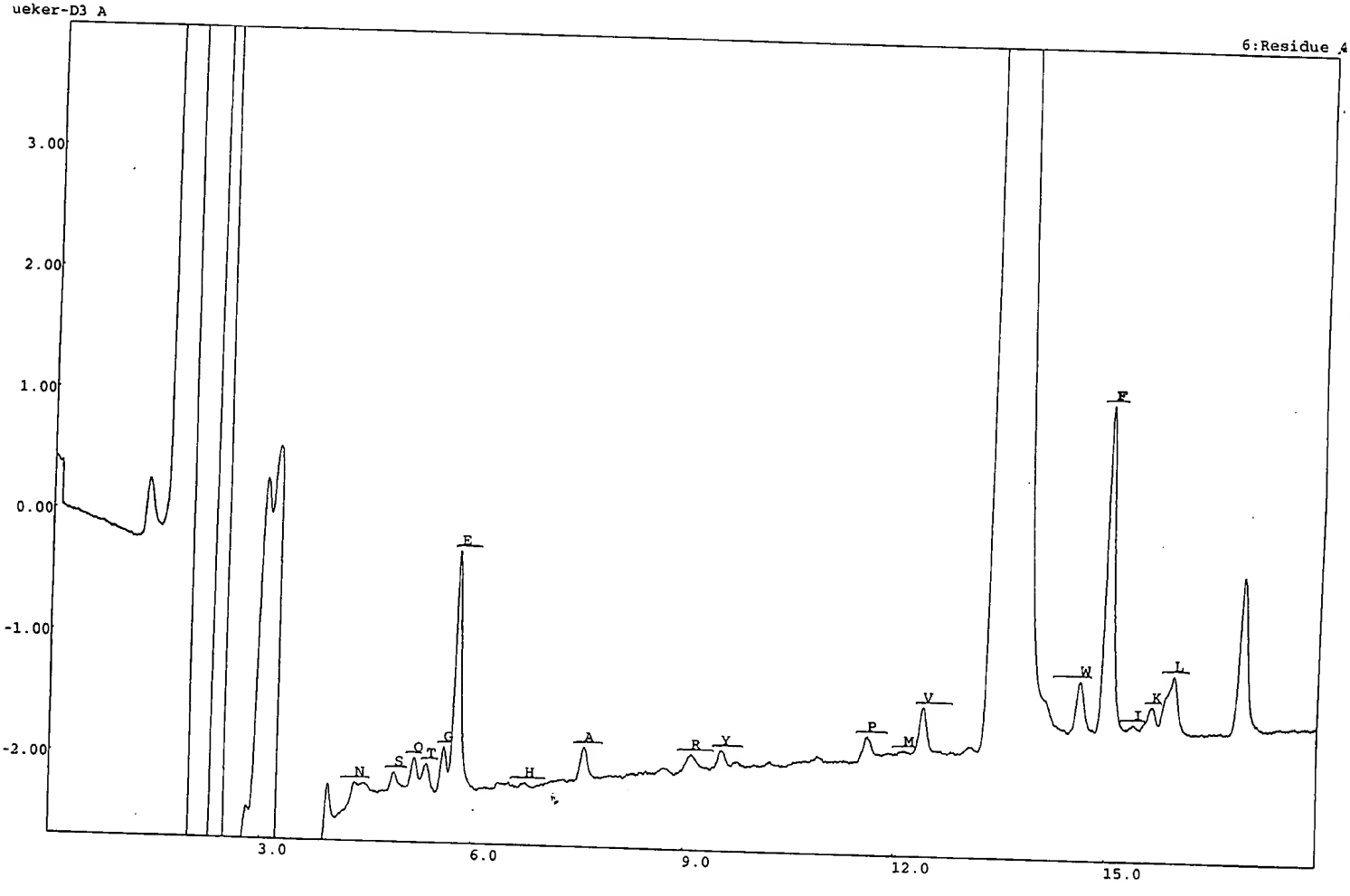

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EXHIBIT C

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University of Agricultural Sciences

ao. Univ. Prof. Dipl. Ing. Dr. Florian Rüker

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A-1190 Vienna, Austria
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e-mail: ruker@mail.boku.ac.at

To
Dan Carter
NCP

001 256 461 4224

Hi Dan,

the brochure looks good, is informative and it's easy to grasp the message. On the front page, left: '...NCP's staff can support our customers...': shouldn't it say 'its customers'? In the price list: 'Domain III' rather than Domain IIII'. You could give the boundaries of domain I / II and II / III as well (1-385 and 189-585, resp.) if you like.

We got N-terminal sequences of domains I, II and III which were cloned exactly as the previous HSA version in question. Actually, we did not have the HSA itself sequenced, but it could be done anytime. I would estimate that the N-terminus of the HSA should most likely resemble that of Domain I. Due to the restriction site used for cloning, all constructs have a leading EF, and due to unprecise removal ot the α-factor leader sequence by the Pichia cells, part of the molecules have additional EA or A N-terminally to that. Here are the results for the domains in detail:

```
Domain I:   80 % EAEFDAH...,    20%  EFDAH...
Domain II:  80%  EFGKA...,      20%  AEFGKA...
Domain III: 66%  EAEFVEE...     33%  EFVEE...
```

We got the sequence of the HSA secreted with its own leader, and only the correct N-terminus (DAH...) was detected.

I also checked on the remaining proteins in the fridge and here are the approximate amounts that are left:

DOM I: max 5 mg
DOM II: 20 mg), 150 mg), 30 mg (
DOM III: 13 mg 27 mg ( ), 40 mg)
DOM I / II: 170 mg :
1B2A2B: 20 mg)
2A: 10 mg (

All these constructs are with the α-factor leader sequence, therefore N-terminus will be heterogenous, as above. DOM II / III has not been cloned yet.

We seem to have a problem expressing the two new mutants, so far we couldn't find a well expressing Pichia clone. Could it be that these mutations are doing something bad to the structure of the protein, maybe making it susceptible to proteolysis? Anyway, we keep on screening more clones, and I will keep you updated.

Schwab is working with our construct, and hopes to have results by the end of the month.

Best regards,

Florian